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Review

The switch helix: A putative combinatorial relay for interprotomer communication in cGMP-dependent protein kinase ☆

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ABSTRACT

For over three decades the isozymes of cGMP-dependent protein kinase (PKG) have been studied using an array of biochemical and biophysical techniques. When compared to its closest cousin, cAMP-dependent protein kinase (PKA), these studies revealed a set of identical domain types, yet containing distinct, sequence-specific features. The recently solved structure of the PKG regulatory domain showed the presence of the switch helix (SW), a novel motif that promotes the formation of a domain-swapped dimer in the asymmetric unit. This dimer is mediated by the interaction of a knob motif on the C-terminal locus of the SW, with a hydrophobic nest on the opposing protomer. This nest sits adjacent to the cGMP binding pocket of the B-site. Priming of this site by cGMP may influence the geometry of the hydrophobic nest. Moreover, this unique interaction may have wide implications for the architecture of the inactive and active forms of PKG. This article is part of a Special Issue entitled: Inhibitors of Protein Kinases (2012).

1. Introduction

All PKG isoforms are essential signaling components in virtually every mammalian tissue. As members of the AGC family of basophilic protein kinases, PKG type I and II isoforms serve as the principle intracellular receptors for the second-messenger cyclic 3′,5′-guanosine-monophosphate (cGMP) [1]. In a fashion thought to be similar to PKA activation, cGMP binds to the regulatory domain at two sites to promote activation of the protein kinase [2,3]. However, several factors challenge a simple description of the structural events that lead to PKG holoenzyme activation, including the order of the high and low-affinity cyclic nucleotide-binding sites, unique structural and biochemical differences in the dimerization domains of PKG and PKA, and the feature that the catalytic domain of PKG is linked to its regulatory domain in the same polypeptide chain.

Remarkably, the type I and type II PKG isoforms share an overall identical domain organization consisting of N-terminal regulatory domains and C-terminal catalytic domains (Fig. 1) [4,5]. Alternative splicing of the type Iα and Iβ isoforms provides the largest deviation from sequence homology [6–8]. Type II PKG results from a different gene altogether, and again shows limited homology to type I [9]. Because of these amino acid sequence differences, it has been proposed that residues from the dimerization and autoinhibitory domains are largely responsible for the kinetic phenotype of the PKG isoforms (Fig. 2a) [10–12]. Following the autoinhibitory domain, all PKG isoforms are nearly identical in amino acid sequence. First, the regulatory domains carry two in-tandem cGMP binding sites A and B (Fig. 2b) [2,4]. In contrast to PKA, the N-terminal A site is the high affinity binding site and the C-terminal B site the low affinity site in PKG [13,14]. It has been a particular challenge to understand these compositional differences between PKA and PKG in terms of their contributions to the molecular mechanisms of kinase activation. In addition, the catalytic domain resides C-terminal to the low affinity cGMP-binding site B with minimal sequence overlap and homology to PKA [15].

In an attempt to shed light on the molecular mechanisms of PKG activation, recent crystallographic studies have examined the isolated N-terminal dimerization domain (DD) (Iβ, 5–55), the isolated high affinity cGMP binding site (Iβ, 92–227) as well as the majority of the regulatory domain (Iα, 78–355) [16–18]. In PKG, like that of PKA, the N-terminal DD provides the structural-basis for homodimer formation. This alignment has been repeatedly confirmed in both type I isoforms by multiple biophysical techniques; however, the only high-resolution structure available is derived from the Iβ isoform and confirms previous experimental examination of the Iα isoform [18–21]. Examination of the latter constructs revealed that the A-site of type I PKG isoforms could bind both cAMP and cGMP, and the identification of a previously uncharacterized domain called the switch helix (SW) that promotes dimerization between protomers. The discovery of these unexpected interactions may prove valuable in discerning the molecular events that are crucial for domain reorganization accompanied during PKG holoenzyme activation.

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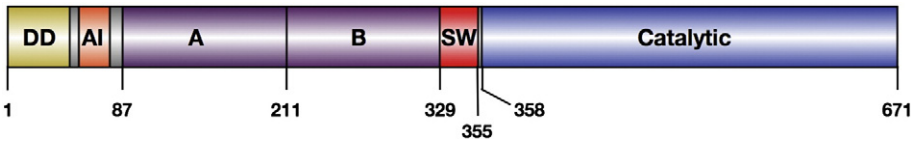

Fig. 1. Domain architecture of PKG1α from *Bos taurus* showing the placement of the dimerization domain (DD), autoinhibitory fragment (AI), and regulatory (A/B), and catalytic domains.

2. Choosing symmetry-related solutions to the crystal structure of PKG Iα (78–355)

The structure of the homodimeric form of the PKG Iα regulatory subunit detailed a new paradigm for considering PKG holoenzyme formation [17]. PKG 78–355 crystals diffracted to 2.5 Å and grew in the C2 space group. The asymmetric unit contained two protein molecules related by non-crystallographic symmetry along a 2-fold axis for which electron density was resolved for the entire construct. The orientation of dimerization was chosen by energetic analysis of the two prominent dimers in the asymmetric unit (Fig. 3). Independent PDBe/PISA analysis found that, although the iso dimer (presented by the two regulatory subunits interacting in an anti-parallel fashion directly via the A- and B-sites, Fig. 3A) had an interaction area of 4640 $Å^2$ (almost 2-fold greater than the SW model), the free energy of interaction was more favorable for the SW dimer (Fig. 3B) [22]. The iso dimer had a free energy calculation of −6.6 kcal/mol (ΔG^{int}), requiring 9.6 kcal/mol for dissociation (ΔG^{diss}). The SW model buries 2740 $Å^2$ with ΔG^{int} calculated at −26.4 kcal/mol and a ΔG^{diss} of 15.8 kcal/mol. Further investigation of these two dimers examined the p-value associated with the interaction free energy. These values suggested the iso dimer interface ($p = 0.890$) was indicative of an interaction having been formed as a consequence of crystallization, while the SW model ($p = 0.027$) likely formed from biologically-relevant contacts. Taken together, this is a strong suggestion that the SW model may represent a physiologically significant macromolecular assembly. The more favorable energetics of the SW model stem from a distinct set of interactions between residues at the C-terminal end of the PKG 78–355, which sit in a nest of hydrophobic residues from the B-site of the opposing symmetry mate. This interaction is further strengthened by a specific hydrogen bond between the backbone carbonyl of T220 and the side chain amino group of N353. No such hydrophobic or specific interactions are observed in the anti-parallel orientation of the iso dimer model.

3. The SW-helix as a novel means of allosteric regulation

The structure of the regulatory subunit of PKG Iα revealed a unique interaction site at the C-terminal tail of the SW helix motif which we termed the "knob/nest" (Fig. 4). The knob motif is located at the C-terminus of the SW helix on the opposing protomer and is composed of the conserved residues FFANL, which is present in both PKG Iα and Iβ, but not in PKG II (Fig. 4b). The nest site is a sub-domain of the cyclic nucleotide B-site (Fig. 4a) and is composed of hydrophobic residues originating from the N3A motif, αB, β6, and the phosphate-binding cassette (PBC). The nest directly abuts the cGMP binding site and is partly formed by β6 and the PBC (Fig. 5a). The knob/nest interaction in solution was also supported by deuterium exchange mass spectrometry (DxMS) wherein we observed partial protection of C-terminal tail residues that encompassed the knob motif [17]. This further strengthens the putative physiological significance of the SW dimer predicted by PDBe/PISA.

The majority of the interactions provided by the knob motif with the nest residues are between F350, F351 and L354, which encompass 1332 $Å^2$ of the buried surface area out of a total of 2740 $Å^2$ for the SW dimer (Fig. 5b). Although the B-site is unoccupied by cyclic nucleotide, when compared to the previously reported A-sites from PKG Iβ that contain cyclic nucleotide, a structural alignment reveals that there is a 4.8 Å shift in the PBC associated with cGMP binding but only 3.3 Å with cAMP (Fig. 6) [16]. The alignment of the β-barrel of these structures displayed an RMSD of less than 1 Å.

The conformational state of the cGMP binding site in the B-domain and its contiguous nature with the hydrophobic nest may suggest an

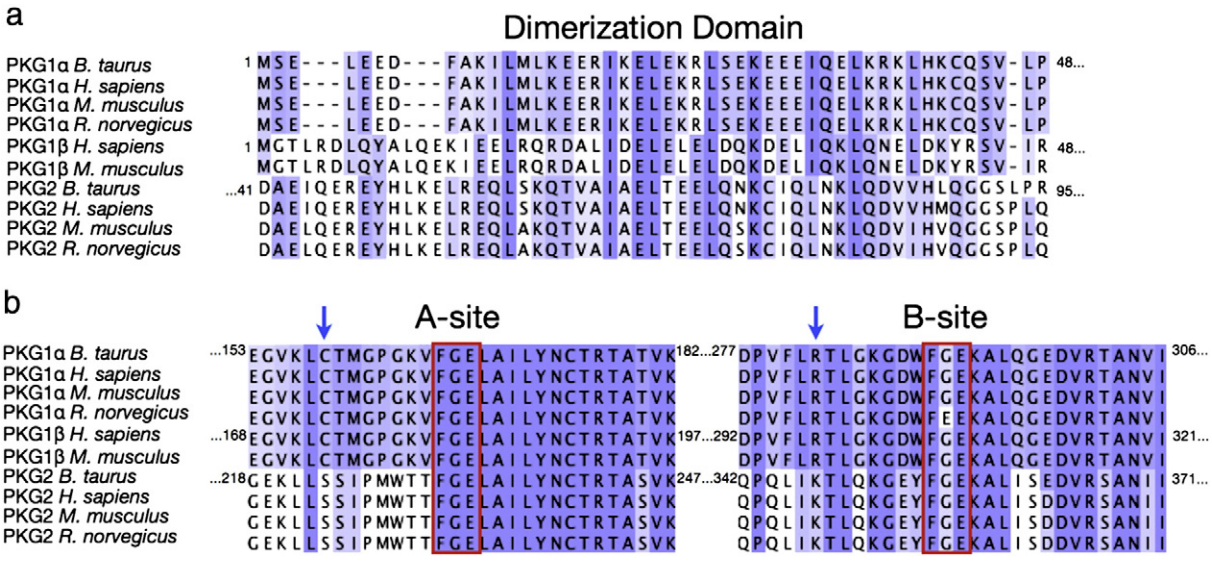

Fig. 2. A multiple sequence alignment colored by BLOSUM62 score of PKG I and II isoforms. a) The conservation of leucine and isoleucine residues for the dimerization domain constructed of a leucine-zipper motif. b) The sequence conservation of the nucleotide binding sites for the high-affinity A-site and the low affinity B-site is shown. The phosphate-binding cassettes – highlighted in red – are highly conserved sites involved in binding of the cyclic nucleotide within the two sites. In type I isoforms, both sites are wholly conserved. There is divergence between type I and II isoforms between the sequences of the two sites, although the B-site is more highly conserved across all isoforms.